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Phosphate fertilizers increase CO$_2$ assimilation and yield of soybean in a shaded environment

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Abstract

Two light treatments [ambient sunlight (L1) during the entire growth period and 40% shade (L2) from 40 d after sowing until 24 d after flowering] and two phosphate fertilizer treatments [no phosphate fertilizer application (P0) and a conventional phosphate fertilizer application (P1)] were used to determine how phosphate fertilizer regulates soybean [*Glycine max* (L.) Merr.] photosynthesis under shading. We showed that phosphorus significantly increased chlorophyll content and grain yield under shading. The light-saturated net photosynthetic rate, apparent quantum yield, maximum electron transport rate, and maximum Rubisco carboxylation rate in P1 under L2 significantly increased. Moreover, phosphate fertilizer significantly improved the electron transfer and PSII reaction center performance under shading. Therefore, phosphate fertilizer increases low light-utilization efficiency by improving PSII performance, promoting ribulose-1,5-bisphosphate regeneration, ensuring a source of carboxylate substrates, and coordinating the balance between photochemical reaction and Calvin cycle under shading.

Keywords: phosphate fertilizer; photosynthesis; shading; soybean; yield.

Introduction

Soybean is one of the most important crops in the world and is native to China. Many factors affect the soybean production scale, of which the low yield is one of the most prominent factors. It has been reported that leaf photosynthetic capacity has the potential to improve biomass productivity in crop plants (Long *et al.* 2006).

Highlights

- Phosphorus improved light absorption and utilization of soybean leaves under shading
- Phosphorus increased the assimilation force for the Calvin cycle under shading
- Phosphorus quickly restored the balance of light and dark reactions after re-lighting

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Abbreviations: AQY – apparent quantum yield; Chl – chlorophyll; C_i – intercellular CO$_2$ concentration; FBPA – fructose-1,6-bisphosphate aldolase; F_v/F_m – maximum quantum yield of PSII primary photochemistry; I – light intensity; I_c – light-compensation point; I_{sat} – light-saturation point; J_{max} – maximum electron transport rate; OJIP – Chl *a* fluorescence transient curve; PI$_{ABS}$ – performance index on an absorption basis; P_N – net photosynthetic rate; P_{Nmax} – light-saturated net photosynthetic rate; P_N–C_i – net photosynthetic rate–intercellular CO$_2$ concentration curve; P_N–I – net photosynthetic rate–light intensity curve; R1 – beginning of flowering; R3 – beginning of the pod stage; R5 – beginning of seeding; R_D – dark respiration rate; RuBP – ribulose-1,5-bisphosphate; SBPase – sedoheptulose-1,7-bisphosphatase; V_{cmax} – maximum ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) carboxylation rate; V_j – relative variable fluorescence at 2 ms; W_k – relative variable fluorescence at 300 µs; φ_{Eo} – quantum yield of electron transport; ψ_o – probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-.

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As one of the most important environmental factors in agricultural production (Yang *et al.* 2014), light is a driving force that affects the structure and function of the photosynthetic mechanisms (Jiang *et al.* 2011, Li *et al.* 2014). Ambient light intensity that is persistently or during a short term significantly lower than the light-saturation point, but not lower than the lowest light intensity that restricts plant survival, is called low light adversity (*see* 'Discussion'). In the past half-century, the number of sunshine hours and solar radiation for crops has persistently decreased due to the increase in the concentrations of aerosols caused by human activities and the associated light fog and low clouds, particularly in the Huanghuaihai Plain of East China during middle August to middle September (30 ± 5 d), which severely restricts soybean production (Ren *et al.* 2005).

Photosynthesis is a complex biochemical and biophysical process comprised of the synthesis of photosynthetic pigment, transport of light-energy electrons, and the Calvin cycle (Wang *et al.* 2013, Yang *et al.* 2018). These processes are all affected by light intensity (Gommers *et al.* 2013). The general increase in leaf area, chlorophyll (Chl) content, and antenna pigment–proteins in a shaded environment increases light interception and absorption efficiency (Melis and Harvey 1981, Evans and Poorter 2001). However, the decrease in light energy conversion and electron transfer efficiency (Yang *et al.* 2017, Huang *et al.* 2018), as well as the expression level and activity of photosynthetic carbon immobilizes enzymes (Sun *et al.* 2014, Liu *et al.* 2017a, Gao *et al.* 2020), which suppresses the photosynthetic rate. Increasing nutrient content is one way to improve the light-utilization ability of crops (Yao *et al.* 2016). Phosphorus regulates the stability of the thylakoid membranes and the activity of photosynthetic proteins penetrating inside and outside the membrane (Liu *et al.* 2017a, Bhattacharya 2019), thereby affecting photosynthetic electron transport, photosynthetic phosphorylation, the Calvin cycle, assimilation, transport efficiency (Singh *et al.* 2018), and photosynthesis. Soybean requires a large amount of phosphorus; thus, a reasonable application of phosphorus fertilizer is an important measure to regulate soybean growth and yield (Taliman *et al.* 2019).

Previous reports have focused on light intensity or phosphorus as a single factor to increase photosynthetic capacity and the physiological mechanisms involved in increased yield (Singh *et al.* 2018, Fan *et al.* 2019, Taliman *et al.* 2019). According to field test results, a limited linear relationship exists between the amount of phosphorus applied and the photosynthetic rate. Photosynthesis decreases rather than increases when the amount of phosphorus applied exceeds a certain ratio (Zhang *et al.* 2018), indicating that light and phosphorus have complementary effects. Maize growers can maximize leaf area and the net photosynthetic rate to attain high grain yields by properly managing P fertilization (Zhang *et al.* 2018). Managing P fertilization also increases photosynthetic carbon and nitrogen metabolism, as well as water-use efficiency of dwarf bamboo under drought stress (Liu *et al.* 2015, 2017b). Based on the effect of phosphate fertilizer on the photosynthetic performance of plants in a stressful environment, we speculated that phosphate fertilizer could promote soybean photosynthesis in a shaded environment. We hypothesized that phosphate fertilizer increases the electron transfer rate of the PSII reaction center, the Rubisco carboxylation rate, and the ribulose-1,5-bisphosphate (RuBP) regeneration rate to compensate for the decrease in light intensity in a shaded environment, thereby increasing the photosynthetic rate.

Few reports are available on the correlation between phosphorus and the relatively poor utilization efficiency of light in the shade, and the complementary mechanism remains uncertain. The consequence of phosphate fertilizers compensating for a decrease in light intensity and an increase in photosynthesis needs to be further clarified. Thus, this experiment focused on exploring the compensatory effect of phosphorus on the reduction of light intensity in a shaded environment, as well as the mechanism by which phosphate fertilizers increase the photosynthetic capacity of soybeans in a shaded environment. We expect to provide a theoretical basis for innovating the agricultural production model for Huanghuaihai Plain (East China) summer soybean with efficient use of low light. If the phosphorus fertilizer improves the net photosynthetic rate (P_N) significantly in a shaded environment, the N, P, and K fertilizer ratio can be adjusted for soybean culture in this region to appropriately increase P fertilizer and reduce N input (we need to design more experiments to prove this supposition). Therefore, the soybean yield (under low light) can be improved, under the normalization of low light conditions from August to September in this area.

Materials and methods

Plant material: The Qihuang 34 soybean hybrid was approved by the National Crop Variety Examination and Approval Committee of China in 2013 and 2018, its main planting areas are Huanghuaihai middle plain and north plain.

Field location: A field experiment was conducted during 2019 at the Agronomy Station of Shandong Agricultural University, Tai'an City, Shandong Province (117°09'E, 36°09'N), which has a typical temperate continental monsoon climate with an annual average temperature of 13.6°C and an annual average rainfall of 600.75 mm. The precipitation and atmospheric temperature dynamics in 2019 are shown in Fig. 1. The major initial properties within the 0–20 cm soil depth were: 12.53 g(soil organic carbon) kg^{-1}, 1.15 g(soil total nitrogen) kg^{-1}, 11.27 mg(available phosphorus) kg^{-1}, 0.68 g(total phosphorus) kg^{-1}, 80.9 mg(available potassium) kg^{-1}, and pH of 6.8.

Experimental design: Two light treatments (L1: ambient sunlight; L2: 40% shade) and two phosphate concentrations [P1: 0 kg(P_2O_5) ha^{-1}; P2: 180 kg(P_2O_5) ha^{-1}] were arranged in a field under a randomized block design. The shade treatment was applied from 40 d after sowing to 24 d after flowering, which lasted 31 d, followed by

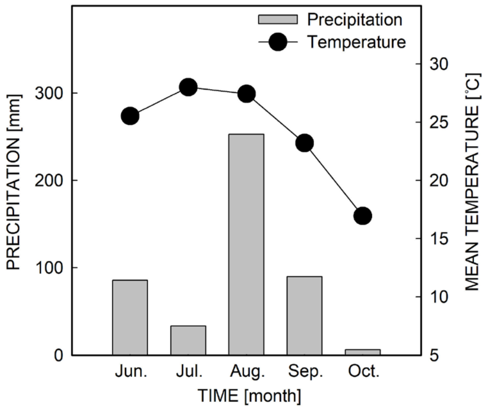

Fig. 1. Precipitation and mean temperatures during the 2019 cropping season. Bars indicate precipitation and line indicates the mean monthly temperature.

sunlight exposition. Shade tents were constructed with commercially available shade cloth (*Hongda Shade Cloth Co.*, Shouguang, China) and scaffolding was used to hold the cloth about 1.5 m above the crop. Each plot was 35 m^2 (5 × 7 m), and three replicates were included in the experiment. The L1 and L2 light intensities are shown in Fig. 1S (*supplement*). Qihuang 34 was selected as the plant material in this study with a planting density of 120,000 plants ha^{-1}. The measurements were made on the inverse fourth leaf at the beginning of the flowering (R1), the beginning of the pod (R3), and the beginning of the filling stage (R5). Seeds were sown on 17 June 2019, and the plants were harvested on 15 October 2019. N and K were applied with 300 kg(urea) ha^{-1} (N content was 46.7%), 300 kg(potassium sulfate) ha^{-1} (K_2O content was 50.0%), and calcium superphosphate, P_2O_5 content was 12%, as P fertilizer. P and K fertilizer were applied as the base fertilizer. Half of the N fertilizer was applied at sowing and the other half at the beginning of the pod stage. Water was supplied throughout the growth period. Disease, weeds, and pests were well controlled in each treatment.

Crop yield was measured at maturity as follows: (*1*) in each plot, the soybean pods were hand-harvested from two rows that were 10 m long; (*2*) the samples were air-dried, threshed, the grain water content was determined after oven-drying at 60°C for 96 h, and adjusting the respective masses to 13% moisture.

Chlorophyll *a* and *b* measurements: Chlorophyll (Chl) *a* and *b* were extracted with 80% acetone from inverse fourth leaf soybean leaves (fully expanded and exposed) at R1, R3, and R5 in each treatment group. The absorbance of the extract was measured with a *UV-2450* spectrophotometer (*Shimadzu Suzhou Instruments Manufacturing Co., Ltd.*, Suzhou, China) at 663 (A_{663}) and 646 nm (A_{646}), respectively. Chl *a* and *b* contents were calculated using equations established by Lichtenthaler and Wellburn (1983): Chl *a* [$\mu g\ mL^{-1}$] = $12.21\ A_{663} - 2.81\ A_{646}$, Chl *b* [$\mu g\ mL^{-1}$] = $20.13\ A_{646} - 5.03\ A_{663}$.

Gas-exchange measurements: Gas exchange was measured using a portable gas-exchange system (*CIRAS-3*, *PP System Ltd.*, Ayrshire, UK). The measurements were made on the inverse fourth leaf at R1, R3, and R5. The responses of P_N to intercellular CO_2 concentration (C_i) were measured at a leaf temperature of 25°C, and a light intensity of 1,400 μmol(photon) $m^{-2}\ s^{-1}$. Plants were acclimated to these conditions until steady-state gas exchange was reached (20–30 min), and net photosynthetic rate–intercellular CO_2 concentration curves (P_N–C_i) were measured with a sequence of reference CO_2 concentrations of 400, 350, 300, 250, 200, 150, 100, 75, 50, 400, 400, 400, 400, 500, 550, 600, 700, 800; 1,000, 1,200; and 1,400 μmol mol^{-1}. The middle four measurements at 400 μmol mol^{-1} were disregarded, as their purpose was to allow the plant to reacclimate to this condition after being exposed to a low CO_2 concentration. Data points were taken in sequential order with an equilibration time of 180–300 s at each CO_2 concentration. Light-saturated net photosynthetic rate (P_{Nmax}) was measured on the inverse fourth leaf at R1, R3, and R5, a CO_2 concentration of 400 μmol mol^{-1}, leaf temperature of 25°C, and light intensity of 1,400 μmol(photon) $m^{-2}\ s^{-1}$.

Net photosynthetic rate–light intensity curves (*P_N–I*): P_N–*I* curves were prepared using a portable gas-exchange system (*CIRAS-3*, *PP Systems*, Amesbury, MA, USA). Measurements were made on the inverse fourth leaf of R1, R3, and R5. The response of the P_N to light intensity (*I*) was measured at a leaf temperature of 25°C, relative humidity of 50–60%, and a CO_2 concentration of 400 μmol mol^{-1}. Leaves were initially stabilized at a saturating irradiance of 2,000 μmol(photon) $m^{-2}\ s^{-1}$, and P_N was measured at PPFD of 50, 100, 150, 200, 400, 800; 1,200; 1,600; and 2,000 μmol $m^{-2}\ s^{-1}$. The measurements were recorded after P_N reached a new steady-state (1–2 min) before changing to a new light level. The calculations for the dark respiration rate (R_D), apparent quantum yield (AQY), light-compensation point (I_c), and saturation intensity (I_{sat}) were taken from Ye *et al.* (2013).

Chlorophyll fluorescence: Chl fluorescence measurements were made using the *Handy PEA* fluorometer (*Hansatech Instruments Ltd.*, King's Lynn, Norfolk, UK). Measurements were made on the inverse fourth leaf of R1, R3, and R5. The leaves were dark-adapted for 20 min using leaf clips. The 4-mm diameter dark-adapted leaf samples within each clip were illuminated with 660-nm light of 3,500 μmol(photon) $m^{-2}\ s^{-1}$ for 1 s. The descriptions and equations for calculating the JIP-test parameters are explained in the Appendix.

Statistical analysis: *Excel 2016* (*Microsoft Inc.*, Redmond, WA, USA) was used to process the raw data. Analysis of variance (*ANOVA*) was performed using *SPSS 16.0* software (*SPSS Inc.*, Chicago, IL, USA). Significant differences between treatments were assessed by *Duncan*'s test. A *P*-value<0.05 was considered significant. The illustrations were prepared *via Sigmaplot* (*ver. 10.0*, *Systat Software*, *Inc*., San Jose, CA, USA).

Results

Phosphorus significantly improves dry matter accumulation and yield of shaded soybean plants: Soybean yield increased significantly after phosphorus was applied under the same light conditions (Table 1). Soybean yield decreased significantly under the shade conditions, with a trend of L1P1 > L1P0 > L2P1 > L2P0. The light treatment had a significant effect on 1,000-seed mass, effective pods per plant, and seeds per plant. The L2 treatment significantly decreased the number of effective pods per plant, seeds per plant, and yield. The phosphate fertilizer treatments only had a significant effect on yield, P1 > P0. No significant differences were observed in the number of effective pods per plant or the seeds per plant. The main reason is that the differences inside the treatments were too large, resulting in insignificant differences between the treatments.

The effects of phosphate fertilizer on soybean yield were different under L1 and L2. Two-way *ANOVA* revealed a significant interactive effect between light intensity and phosphorus on effective pods per plant, number of seeds per plant, and soybean yield. Effective pods per plant and the number of seeds per plant were closely related to light ($P<0.01$) and phosphorus ($P<0.01$) and the interaction between light and phosphorus ($P<0.01$).

The 1,000-seed mass was significantly different between L1 and L2, which was 9.2% but it was not significant between P0 and P1. Effective pods per plant and the number of seeds per plant in L2 decreased) 41.1 and 35.9%, respectively, compared to L1 but increased by 16.4 and 31.3%, respectively, for P0 and P1. The yield of the P1 treatment increased by 20.2 and 34.2% compared to P0 under the L1 and L2 treatments, respectively. Soybean yield increased significantly after applying phosphate fertilizer in the shade, and the increase was significantly higher than that under full-light conditions. The phosphate fertilizer promoted flowering and pod formation in soybean, which increased the yield by promoting an increase in the number of effective pods per plant and the number of seeds per plant.

Phosphorus fertilizer increases the Chl content of plant leaves under shade: Two-way *ANOVA* revealed that Chl content was closely associated with light ($P<0.05$) and phosphorus ($P<0.05$), but it did not significantly affect the interaction between light and phosphorus. Chl *a* and *b* content at each growth stage after anthesis increased significantly with the decrease in light intensity and application of phosphate fertilizer (Fig. 2). Shading was the principal reason for the significant increase in soybean Chl content. Phosphorus fertilizer had different effects on Chl content under different light environments. For instance, the Chl content of the L1P1 treatment increased by 9.6 and 16.1%, respectively, compared to the L1P0 treatment, which was significantly less than 13.8 and 21.8% under the shade environment conditions; however, Chl *b* increased by 16.8 and 29.3% and 23.2 and 24.9% under the L1 and L2 treatments after phosphate fertilizer was applied during the R3 and R5 growth stages,

Table 1. Effects of different treatments on yield and summer soybean components. L1 – ambient sunlight; L2 – 40% shade; P0 – phosphate fertilizer application of 0 kg(P_2O_5) ha^{-1}; P1 – phosphate fertilizer application of 180 kg(P_2O_5) ha^{-1}. L – light factor; P – phosphate fertilizer factor; L × P – interaction between light and phosphate fertilizer. Data are means of three replications. *Different lowercase letters* in the same column indicate significant differences ($P<0.05$). *$P<0.05$; **$P<0.01$; ns – not significant.

Treatment Light (L)	Phosphorus (P)	1000-seeds mass [g]	Effective pods per plant	Seeds per plant	Yield [kg ha^{-1}]
L1	P0	276.2 ± 9.1	66.9 ± 2.0	117.2 ± 1.7	3,611.5 ± 12.5
	P1	272.6 ± 2.7	77.7 ± 2.0	150.9 ± 2.5	4,340.7 ± 115.9
L2	P0	298.1 ± 2.6	39.2 ± 1.4	73.0 ± 1.4	2,451.6 ± 100.2
	P1	301.2 ± 1.2	45.9 ± 1.2	98.8 ± 1.9	3,291.0 ± 44.1
Mean for light (L)					
L1		274.4^b	72.3^a	134.1^a	$3,976.1^a$
L2		299.7^a	42.6^b	85.9^b	$2,871.3^b$
Mean for phosphorus (P)					
P0		287.2	53.1	95.1	$3,031.6^b$
P1		286.9	61.8	124.9	$3,815.9^a$
Significance					
L		**	**	**	**
P		ns	**	**	**
L × P		ns	**	**	**

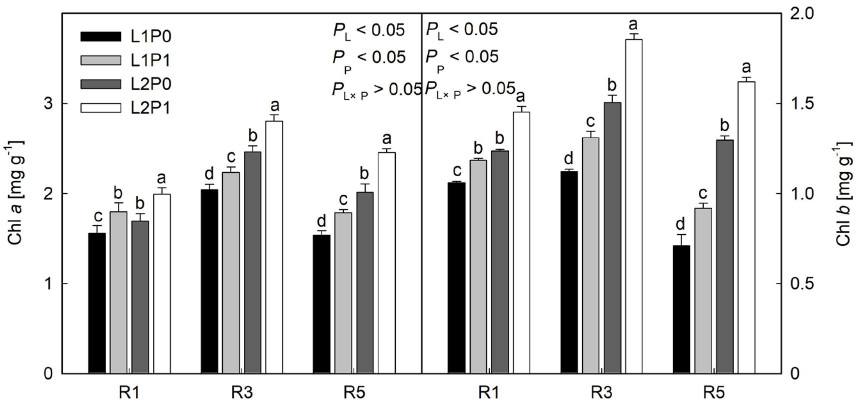

Fig. 2. Changes in chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) contents of the different treatments. L1 – ambient sunlight; L2 – 40% shade; P0 – phosphate fertilizer application of 0 kg(P_2O_5) ha^{-1}; P1 – phosphate fertilizer application of 180 kg(P_2O_5) ha^{-1}. R1 – beginning of flowering; R3 – beginning of the pod stage; R5 – beginning of seeding. Data are means of nine replications. *Lowercase letters* indicate significant differences between the different treatments (P<0.05, *ANOVA*) at R1, R3, and R5, respectively.

respectively. These results show that applying phosphate fertilizer had a significant effect on Chl content under shading, particularly Chl *b* content.

Phosphate fertilizer improves the light energy-utilization capacity of soybean under the shade: Two-way *ANOVA* revealed that P_{Nmax} was closely associated with light and phosphorus, but it did not significantly affect the interaction between light and phosphorus. P_{Nmax} was determined to detect the change in the photosynthetic rate under the shade conditions. The P_{Nmax} value during the R1 period after shading was significantly lower than that in the L1 treatment (Fig. 3). In L2P0, the P_{Nmax} value decreased by 38.3% and that of L2P1 decreased by 26.3% under the same phosphate fertilizer treatment. The CO_2 assimilation rate of the P1 treatment increased significantly under shading compared to the P0 treatment, as the shading time increased. After re-lighting during the R5 period, L1P1 was 4.6% higher than that of L2P1, indicating that applying phosphorus under the shade condition was beneficial to maintain a high rate of CO_2 assimilation after re-lighting during the later stages of plant growth.

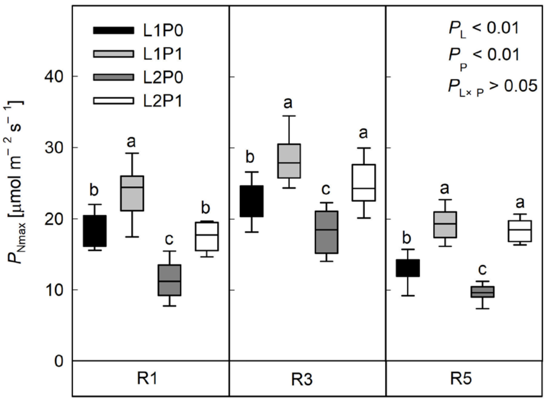

Fig. 3. Changes in light-saturated net photosynthetic rate (P_{Nmax}) of the different treatments. L1 – ambient sunlight; L2 – 40% shade; P0 – phosphate fertilizer application of 0 kg(P_2O_5) ha^{-1}; P1 – phosphate fertilizer application of 180 kg(P_2O_5) ha^{-1}. R1 – beginning of flowering; R3 – beginning of the pod stage; R5 – beginning of seeding. *Lowercase letters* indicate significant differences between the different treatments (P<0.05, *ANOVA*) at R1, R3, and R5, respectively.

To further explore the reasons for the significant increase in the P_{Nmax} value of the L2P1 treatment during the R3 period, net photosynthetic rates were determined as a function of light intensity. The light-response curves revealed that the net photosynthetic rates were significantly lower in the L2 treatment than that in the L1 treatment, showing a trend of L1P1 > L1P0 > L2P1 > L2P0, but as shade treatment time increased, the net photosynthetic rate of the L2P1 treatment gradually increased, and the order became L1P1 > L2P1 > L1P0 > L2P0 (Fig. 4). The L2P1 net photosynthetic rate remained high after re-lighting.

We focused on the response of the net photosynthetic rate to low light intensity [≤ 200 μmol(photon) m^{-2} s^{-1}] to determine the change in AQY under the shade environment. The initial slope of the P_N–I curve reflected the plant's low light-utilization ability. The AQY of plants under low light trended significantly upward, particularly in the L2P1 treatment. I_c and R_D increased significantly in the shaded condition after analyzing I_c (light-compensation point) and R_D (dark respiration rate). The I_c and R_D of the P1 treatment under the two lighting conditions were lower than those of the P0 treatment, and the difference between P1 and P0 in the shaded conditions was more significant (Table 1S, *supplement*). The low light [100 μmol(photon) m^{-2} s^{-1}] utilization capacity of plants under the shade conditions was significantly higher than that under the full-light conditions (Fig. 2S, *supplement*). As adaptability to low light improved, the plant's high light intensity [800 μmol(photon) m^{-2} s^{-1}] utilization capacity also increased in parallel. The low light-utilization capacity of L2P1 plants after re-lighting was high, indicating that applying phosphorus under the shade conditions allowed the plants to adapt quickly to a low-light environment while ensuring the restoration of photosynthetic capacity after re-lighting, the subsequent accumulation of photosynthetic products, and a significant increase in yield.

Phosphate fertilizer increases the rate of CO_2 assimilation under the shade: We also determined P_N as a function of internal CO_2 concentration in the same plants to investigate the changes in the carboxylation system under the different treatments. In L2P0 and L2P1, net photosynthetic rates decreased significantly after the shade treatment. As the plant's adaptability increased, the net photosynthetic rate of L2P1 plants increased significantly during the R3 period and maintained a high level compared to the L2P0 treatment during re-lighting at R5 (Fig. 3S, *supplement*).

Further analysis of the P_N–C_i curves using the equations published by Farquhar *et al.* (1980) indicated that the light environment significantly affected maximum Rubisco carboxylation rate (V_{cmax}) and maximum electron transport rate (J_{max}). Two-way *ANOVA* revealed that V_{cmax} and J_{max} were closely associated with light and phosphorus but did not significantly affect the interaction between light and phosphorus. V_{cmax} and J_{max} were significantly higher under the natural light conditions than under the shade conditions in the order of L1P1 > L1P0 > L2P1 > L2P1, except during the R5 period (Fig. 5). V_{cmax} and J_{max} responded differently to phosphate fertilizer under the two light conditions. The use of phosphate fertilizer had no significant effect on V_{cmax} under the ambient light environment, as L2P1 and L2P0 were significantly different only during the R3 period under shade. J_{max} changed significantly after applying phosphate fertilizer under different light conditions. J_{max} increased by 8.1 and 16.9% in the P1 treatment during the R3 and R5 periods under the L1 treatment compared with the P0 treatment, and by 38.8 and 32.3% in the L2 treatment, respectively. These results indicate that applying phosphate fertilizer significantly improved the RuBP regeneration rate under the shade conditions.

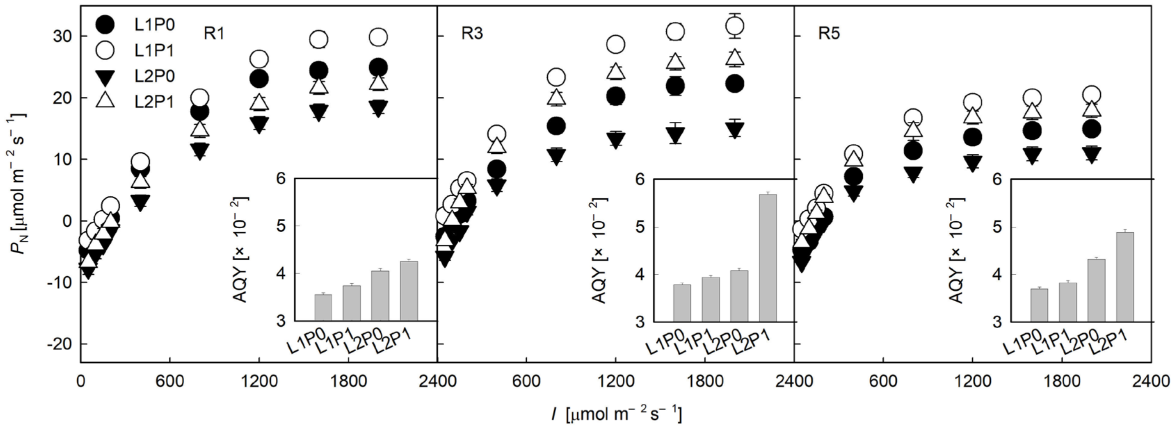

Fig. 4. Changes in net photosynthetic rate–light intensity curve (P_N–I) and apparent quantum yield (AQY) of different treatments. L1 – ambient sunlight; L2 – 40% shade; P0 – phosphate fertilizer application of 0 kg(P_2O_5) ha^{-1}; P1 – phosphate fertilizer application of 180 kg(P_2O_5) ha^{-1}. Data are means of six replications.

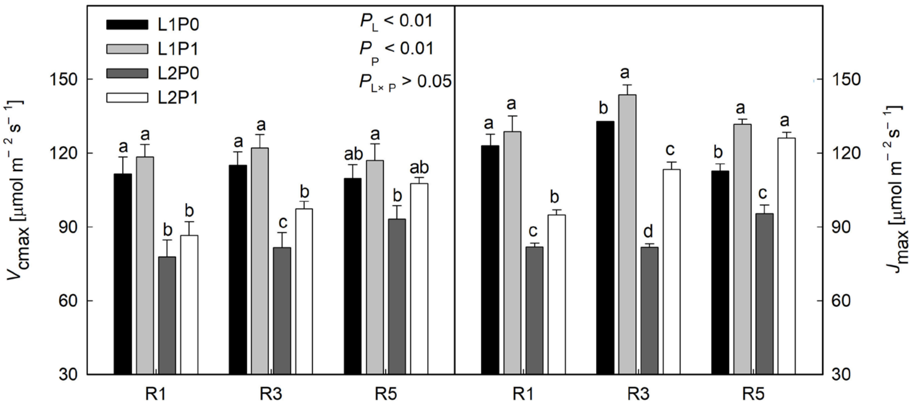

Fig. 5. Changes in maximum ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) carboxylation rate (V_{cmax}) and maximum electron transport rate (J_{max}) of different treatments. L1 – ambient sunlight; L2 – 40% shade; P0 – phosphate fertilizer application of 0 kg(P_2O_5) ha^{-1}; P1 – phosphate fertilizer application of 180 kg(P_2O_5) ha^{-1}. R1 – beginning of flowering; R3 – beginning of the pod stage; R5 – beginning of seeding. Data are means of six replications. *Lowercase letters* indicate significant differences between the different treatments (P<0.05, *ANOVA*) at R1, R3, and R5, respectively.

Chl fluorescence analysis reveals increased photosynthetic efficiency in plants under the shade conditions: Variable fluorescence curves and ΔV_t [for ΔV_t analysis ($\Delta V_t = \Delta(F_t - F_o)/(F_m - F_o)$)] fluorescence in L1P0 treatment on each day of the experiment (was used as a reference and equaled 0) were constructed to compare differences between treatments on each measuring day during the experiment (Fig. 4S, *supplement*). The K- and J-bands revealed significantly higher values for both shade treatments under ambient light, indicating that the PSII donor side and acceptor side were reduced to different degrees under shade, which was alleviated during the R3 period. The J-bands were relatively low after the phosphorus treatment, particularly under the shade conditions, indicating that phosphate fertilizer alleviated the effect of the shade environment on leaf receptor performance.

Fig. 6 shows the change in the relative variable fluorescence intensity at points K and J of the Chl *a* fluorescence transient (OJIP) curves. Two-way *ANOVA* revealed that relative variable fluorescence at 300 µs (W_k) and relative variable fluorescence at 2 ms (V_j) were closely associated with light and phosphorus but did not significantly affect the interaction between light and phosphorus. W_k and V_j increased after the shade treatment, particularly V_j, indicating that the performance of the donor side and the receptor side decreased to varying degrees, and the receptor side was significantly affected by the shade environment. W_k and V_j of phosphorus-applied plants showed a downward trend in the two light treatments. The effect of phosphate fertilizer on W_k was not significant, but V_j decreased significantly. After re-lighting, the L2P1 V_j was significantly lower than that of the L2P0 treatment, but not significantly different from that of the L1P1 treatment. These results indicate that applying phosphorus improved electron transport performance on the receptor side during shade stress and helped retain receptor side performance after the plant was re-lighted.

Analysis of the relative variable fluorescence at points K and J of the OJIP curve revealed that light and phosphate fertilizer had a significant effect on V_j. The change in the relative fluorescence at point J is closely related to the electron transport performance on the PSII acceptor side. Thus, we studied the maximum quantum yield of PSII primary photochemistry (F_v/F_m), the probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (ψ_o), the quantum yield of electron transport (φ_{Eo}), and the performance index on an absorption basis (PI_{ABS}).

F_v/F_m, ψ_o, φ_{Eo}, and PI_{ABS} decreased significantly after the shade treatment (Fig. 7). Two-way *ANOVA* revealed that F_v/F_m, ψ_o, φ_{Eo}, and PI_{ABS} were closely associated with light and phosphorus, but did not significantly affect the interaction between light and phosphorus. The photosynthetic mechanism adapted with improved low light adaptability of the plants. During the R3 period, P1 F_v/F_m, ψ_o, φ_{Eo}, and PI_{ABS} increased by 0.9, 3.4, 9.9, and 20.5% in the L1 treatment compared to the P0, and were 1.5, 6.4, 48.5, and 69.7% in the L2 treatment, respectively. Phosphate fertilizer strengthened PSII performance, particularly under the shade environment. L2P1 had the lowest decrease during the R5 period after re-lighting compared to the other treatments, which was not significantly different from the L1P1 treatment, indicating that applying phosphorus helped maintain the performance of the photosynthetic system after re-lighting, ensured that the absorbed light energy was fully used to promote electron transfer, and improved soybean photosynthetic capacity during a later period.

Discussion

Photosynthesis is the basis of crop growth and yield, but is susceptible to light intensity (Valladares and Niinemets 2008) and decreases significantly under a shaded environment (Li *et al.* 2014). Plants improve the light absorption ability of chloroplasts (Wu *et al.* 2017),

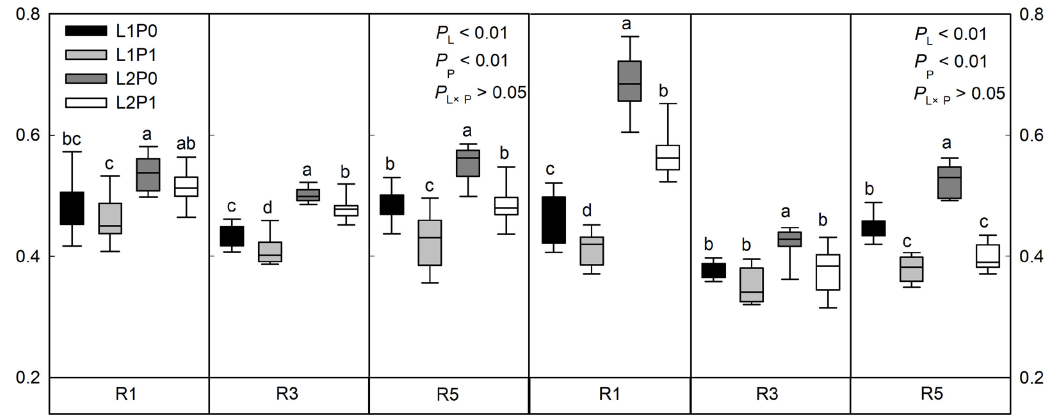

Fig. 6. Changes in relative variable fluorescence at 300 µs (W_k) and relative variable fluorescence at 2 ms (V_j) of different treatments. L1 – ambient sunlight; L2 – 40% shade; P0 – phosphate fertilizer application of 0 kg(P_2O_5) ha^{-1}; P1 – phosphate fertilizer application of 180 kg(P_2O_5) ha^{-1}. R1 – beginning of flowering; R3 – beginning of the pod stage; R5 – beginning of seeding. *Lowercase letters* indicate significant differences between the different treatments (P<0.05, *ANOVA*) at R1, R3, and R5, respectively.

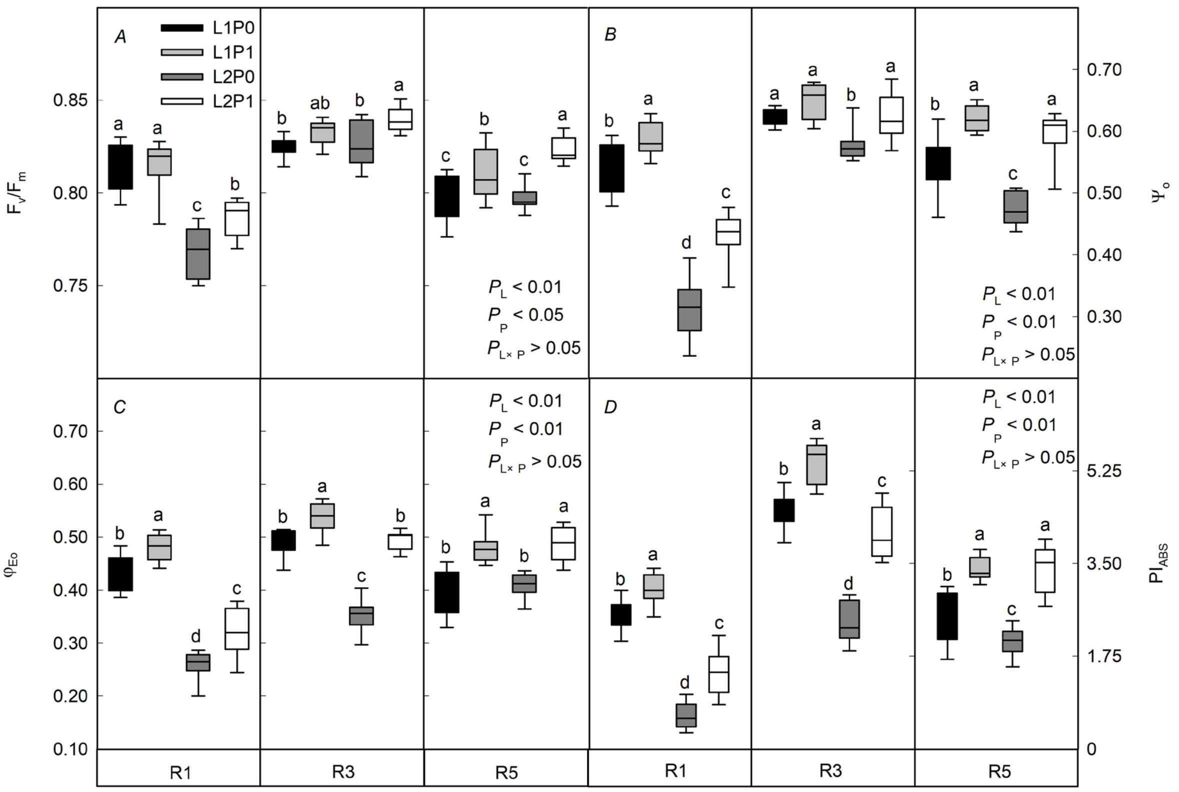

Fig. 7. Changes in the maximum quantum yield of PSII primary photochemistry (F_v/F_m) (*A*), the probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (ψ_o) (*B*), the quantum yield of electron transport (φ_{Eo}) (*C*), and the performance index on an absorption basis (PI_{ABS}) (*D*) of different treatments. L1 – ambient sunlight; L2 – 40% shade; P0 – phosphate fertilizer application of 0 kg(P_2O_5) ha^{-1}; P1 – phosphate fertilizer application of 180 kg(P_2O_5) ha^{-1}. R1 – beginning of flowering; R3 – beginning of the pod stage; R5 – beginning of seeding. *Lowercase letters* indicate significant differences between the different treatments (P<0.05, *ANOVA*) at R1, R3, and R5, respectively.

make full use of low light, and adapt by increasing Chl content. In the present experiment, Chl *a* and *b* increased significantly in the leaves after the shade treatment (Fig. 2), but P_{Nmax} decreased significantly, indicating that the increase in Chl was insufficient to compensate for the decrease in the photosynthetic rate caused by a decrease in light intensity. However, Chl *a*, Chl *b*, and P_{Nmax} of leaves all increased significantly in the P1 treatment, particularly under the shade condition (L2), indicating that the plants increased leaf photosynthetic rate by increasing their light energy capture capacity (Fig. 3). The plants developed an adaptive response after the long shade conditions. AQY is a symbol of the plant's low light usability. Under the shade conditions, the AQY of the plants was significantly higher than that of plants in the ambient light environment and applying phosphorus significantly improved their low light-utilization ability. The AQY in the L2P1 treatment was significantly higher than that of the other treatments during the R3 period (Fig. 4), and the plant's light energy utilization efficiency (P_N/I) increased in parallel [including light intensity of 100 μmol(photon) m^{-2} s^{-1} and light intensity of 800 μmol(photon) m^{-2} s^{-1}] (Fig. 2S), which shows that a reasonable application of phosphorus under a shade condition slowed down the reduction in the CO_2 assimilation rate, quickly improved the plant's adaptability to a shaded environment, and made full use of light energy to produce photosynthetic products, thereby significantly increasing production. R_D and I_c of P1 were lower than that in the P0 treatment, which was more significant in the shaded environment (Table 1S). This result further demonstrates that applying phosphate fertilizer in a shaded environment enhances light energy-utilization efficiency, reduces the light-compensation point, and reduces self-respiratory consumption to adapt to long-term shaded environments. The output of the P1 treatment under the shade condition increased by 34.2% compared to P0 (Table 1), but the phosphate fertilizer only partially alleviated the decrease in the CO_2 assimilation rate and product caused by insufficient light energy.

One question we aimed to address was whether the high CO_2 assimilation rates observed in soybean under the shade conditions were a consequence of the significant improvement in the photosynthetic system or the activities of the related Calvin–Benson cycle. Some studies have shown that C_3 cycle-related enzymes are notably affected by light intensity (Sun *et al.* 2014, Gao *et al.* 2020). As the most abundant enzyme in plants, the proportion of activated Rubisco in C_3 plants is about 25% (Sage and Pearcy 1987, Li *et al.* 2013). This content may be excessive, so the reduction in the Rubisco carboxylation rate can be compensated by rapid activation. Fructose-1,6-bisphosphate aldolase (FBPA), and sedoheptulose-1,7-bisphosphatase (SBPase) are the key enzymes in the C_3 cycle to regenerate RuBP. Small changes in their content have a significant impact on CO_2 assimilation, and activity is affected by light intensity (Raines 2006, Ding *et al.* 2016). The activities of FBPA and SBPase decrease significantly under a shaded environment, affecting RuBP regeneration, and reducing the source of the Rubisco carboxylation substrate (Simkin *et al.* 2017). The P_N–C_i curve showed that carboxylation efficiency was reduced under the shade conditions and was effectively relieved by the phosphorus treatment (Fig. 3S). Further analysis showed that V_{cmax} and J_{max} decreased significantly with a decrease in light. The difference in V_{cmax} between the P0 and P1 treatments was not significant under the same light environment, while J_{max} increased significantly. The increase in the shade condition was significantly higher than that under the ambient light condition (Fig. 5), indicating that phosphate fertilizer applied in the shade mainly increases CO_2 assimilation by increasing the maximum electron transport rate rather than increasing the efficiency of Rubisco carboxylation. This observation also shows that the increase in CO_2 assimilation rate after applying phosphorus during shading may be closely related to improved photosystem performance.

We analyzed PSII based on these results. The primary site of photosynthesis PSII absorbs and converts light energy (Strasser *et al.* 2004, Kalaji *et al.* 2018); its performance is significantly affected by light intensity (Hussain *et al.* 2019). The OJIP curves reflect the process and state of the original photochemical reaction in PSII. F_v/F_m is an important parameter to measure PSII activity under various environmental stressors (Sejima *et al.* 2014). This study discovered changes in F_v/F_m of < 5% before and after applying phosphorus in the L1 and L2 treatments (Fig. 7*A*). Therefore, the effect of applying phosphate fertilizer on the photosynthetic rate of soybean under the shade conditions was very small according to the maximum photochemical efficiency of PSII. However, the relatively variable fluorescence at point J (2 ms) in the OJIP curve of the P0 treatment increased significantly after the shade treatment, compared to the P1 treatment (Figs. 6, 4S), indicating that electron transfer from Q_A to Q_B was obstructed; it means that the PSII receptor-side electron transfer was blocked. We also observed that the relative fluorescence yield of the P0 treatment at point K increased significantly, but not as much as point J, demonstrating that applying phosphate fertilizer improves the activity of the oxygen-evolving complex, but the effect on the smoothness of electron transfer was more significant.

We compared φ_{Eo}, ψ_o, and PI_{ABS} to further explore the effect of phosphate fertilizer on photosystem activity. Studies on the PSII reaction center showed that electron transfer efficiency and light energy absorption performance were significantly reduced under shading (Fig. 7*B–D*). The performance of the receptor side of the plant, electron transfer efficiency, and light energy conversion efficiency of the PSII reaction center were all significantly improved after applying phosphorus fertilizer under the shade conditions, which was consistent with the changes in P_{Nmax}. These results indicate that the increase in the rate of CO_2 assimilation after applying phosphate fertilizer during shading is closely related to improved PSII electron transport performance. The higher performance of the photosystem provides more assimilation force (NADPH and ATP) for RuBP regeneration, and the use of the available photochemical energy to provide Rubisco with a sufficient substrate source.

After resuming light during the R5 period, the V_{cmax} and J_{max} of the L2P1 treatment trended upward, and the performance of the PSII reaction center was minimally reduced. No significant difference in P_{Nmax} was observed compared to L1P1, and light energy utilization efficiency was significantly higher than that of the other treatments. Such results indicate that after restoring the light, plants in the L2P1 treatment used more absorbed light energy for photochemistry due to the restored balance between the light-dependent and light-independent reactions, which maintained the electron transfer efficiency of the photosystem and improved the RuBP-regeneration rate to provide sufficient substrate for Rubisco. This ensured that soybean made full use of the light energy after re-lighting and increased the accumulation rate of photosynthetic product to increase yield.

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Appendix. Selected JIP-test parameters calculated based on fast fluorescence kinetics.

Fluorescence parameters	Description
F_o	Minimal recorded fluorescence intensity
F_m	Maximal recorded fluorescence intensity
$F_j = F_{2ms}$	Fluorescence at 2 ms

$F_k = F_{300\mu s}$	Fluorescence at 300 µs
$F_v/F_m = (F_m - F_o)/F_m$	The maximum quantum yield of PSII primary photochemistry
$V_j = (F_{2ms} - F_o)/(F_m - F_o)$	Relative variable fluorescence at 2 ms
$W_k = (F_{300\mu s} - F_o)/(F_{2ms} - F_o)$	Relative variable fluorescence at 300 µs
$M_o = 4\ (F_{300\mu s} - F_o)/(F_m - F_o)$	The approximated initial slope of the fluorescence transient
$ABS/RC = M_o\ (1/V_j)\ (1/\varphi_{Po})$	Absorption flux per RC
$\psi_o \equiv (1 - V_j)$	The probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-
$\varphi_{Eo} \equiv [1 - (F_o/F_m)]\ \psi_o$	Quantum yield of electron transport
$PI_{ABS} \equiv (RC/ABS)\ [\varphi_{Po}/(1 - \varphi_{Po})]\ [\psi_o/(1 - \psi_o)]$	Performance index on absorption basis